Autolysis of Baker's Yeast[1,2]

DONALD C. VOSTI[3] AND M. A. JOSLYN

Department of Food Technology, University of California, Berkeley, California

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Investigations of the factors influencing yeast autolysis began with Salkowski's accidental observation that sugar accumulated in the supernatant solution of a mixture of compressed baker's yeast with six times its weight of chloroform water solution after storage for a month (Salkowski, 1889). Subsequently, Salkowski (1908) also reported the presence of amino acid in the autolysate, and investigated the possible precursors of sugar liberated (Salkowski, 1921).

Following these early observations, various phases of yeast autolysis were investigated and a considerable literature in this field has accumulated, although in widely scattered publications. The liquefaction, loss in leavening power, and deterioration of baker's yeast has been of great significance industrially. A number of investigations of the autolytic changes occurring in baker's yeast have been made. Equally important, industrially, have been the changes, as a result of autolysis, occurring in the pitching yeast used in breweries. The control of autolytic changes in yeast in the compressed yeast and brewing industry, as well as the utilization of autolysis in the production of yeast nutrients and certain types of wine, particularly the flor sherry wines, has stimulated interest in the general field of yeast autolysis.

Although the general nature of yeast autolysis has been established, our knowledge of the mechanism of autolysis, of the chemical changes involved, and of the structural changes occurring in the yeast cell is still incomplete. The early investigations were carried out largely with poorly described strains of *Saccharomyces cerevisiae* whose conditions of growth, stage of growth, and previous storage were largely unknown. Bacterial contamination, poor control of the degree of acidity (pH) and temperature as well as other environmental conditions were common. The effects of type of yeast and conditions of growth on the rate and course of autolysis were largely not determined. The early methods of analysis, also, were of questionable accuracy. Recent investigations in this field have been scarce.

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[2] Based on data presented in Ph.D. Thesis in Agricultural Chemistry of one of the authors (Donald C. Vosti), University of California, Berkeley, California, June 1952.

[3] Present address: American Can Company, San Francisco, California.

The autolysis of yeast is characterized in general by a latent period during which the yeast cells undergo an active autofermentation, particularly noticeable in glycogen-rich yeast. When the glucose formed from glycogen is exhausted, irreversible changes occur in the cytoplasm leading first to rapid staining and then to characteristic granulation, and then proteolysis occurs, leading to the accumulation of polypeptides and amino acids. Nucleic acids as well as carbohydrates are involved in the early stages of autolysis.

Stacey and Webb (1948) proposed that in the autolysis of gram positive bacteria a change in gram stain precedes lysis in which the relatively labile bond holding ribonucleic acid to a specific polysaccharide is first broken followed by hydrolysis of ribonucleic acid by ribonuclease. Henry and Stacey (1948) offered evidence for the existence of a gram complex between the cell wall and the cytoplasmic membrane in *Saccharomyces cerevisiae*. Lamanna and Mallette (1950) reported that yeast cells are gram positive because of the presence of ribonucleate but did not find that the removal of nucleic acid necessarily resulted in loss of gram stain (see also Webb, 1950, and Bartholomew and Mittwer, 1952).

The influence of antiseptics on the autolysis of yeast was investigated by Navassart (1911), and more recently by Haehn and Leopold (1934a), and Malkov (1950). The effect of pH was investigated by Navassart (1910), Schryver, Thomas and Paine (1927) and particularly by Drews (1936). The production of organic acids, amino acids and carbohydrates during yeast autolysis was reported on by Salkowski (1889), Neuberg and Kerb (1912), Neuberg (1913), Iwanoff (1913), Meisenheimer (1921), and Haehn and Leopold (1937). In recent years, the determination of vitamins liberated in yeast autolysate was investigated by several, particularly Trufanov and Kirsanova (1939, 1940) and Farrer (1946). Proteolytic enzymes in yeast were investigated by Hahn (1898), Geret and Hahn (1898), Cohnheim (1901), Vines (1901, 1902, 1904, 1909), Dernby (1917, 1918a, b), and more recently and extensively by Hecht and Civin (1936), Willstätter and Grassman (1926), Grassman (1927), Johnson and Berger (1940) and Johnson (1941, 1948).

In the older investigations it was indicated that complete liquefaction including lysis of the cell walls occurs during yeast autolysis. The rigidity of the yeast cell

wall, particularly resistance to bursting during freezing was demonstrated by Goetz and Goetz (1938) for *Saccharomyces cerevisiae*. It was reported also by Strain (1939) who noted that completely autolyzed cells retained their normal shape and that it was difficult to differentiate autolyzed from living cells by microscopic observation. The rigidity of the yeast cell wall and its resistance to lysis was noted also in investigations of the microchemistry and electron microscopy of the cell wall at Delft, by Houwink and Kreger (1953).

The effect of the following on the autolysis of suspensions of freshly grown commercial baker's yeast was investigated: density, pH, type and concentration of buffer, toluene, temperature, and various activators and inhibitors. The rate of liberation of total soluble nitrogenous constituents, trichloracetic acid-soluble constituents, free amino acids, ammonia and inorganic phosphate as influenced by the above factors was studied.

Materials and Methods

Freshly pressed baker's yeast, grown under highly aerobic conditions on a molasses mineral salts medium was used for all investigations. This was obtained daily as needed.[4] One pound of the fresh yeast was added to 1.2 liter of heat-sterilized 0.5 M buffer in a 2-liter cotton-stoppered flask and thoroughly mixed by swirling to obtain a uniform slurry. Fifty ml of the suspension were then transferred by sterile pipette into each of 26 to 30 4-oz cotton-stoppered bottles containing 50 ml of sterile buffer. This procedure resulted in a yeast suspension of about 10^9 cells per ml containing about 3 mg of total organic nitrogen per ml. This suspension is of the same order of magnitude as that used by Drews (1936). Preliminary observations indicated that autolysis in more dilute suspensions, 10^6 cells per ml or less, was irregular and difficult to reproduce. The yeast suspension was allowed to autolyze for varying lengths of time up to 72 hours at 53 C $\pm$ 1 C. The cell density (expressed as mg of nitrogen per ml) was determined on each bottle before incubation by withdrawing a 10-ml. sample with a sterile pipette. Two or more bottles of yeast suspension were removed initially and at various time intervals for analysis and observation.

The pH of the entire suspension before and after autolysis and of the cell-free supernatant fluid was measured with glass electrodes with a Beckman electric line operated pH meter standardized against a saturated solution of pure potassium acid tartrate.

The total organic nitrogen content was determined by a micro Kjeldahl procedure on a sample of not over 5 ml containing 0.2 to 2.5 mg of nitrogen after digestion with sulfuric acid-copper sulfate-sodium selenate mixture. This is expressed as total N in mg per ml.

[4] The yeast was generously supplied by the Consumer's Yeast Company of Oakland, California.

The heat coagulable nitrogen was determined by heating 8 ml of the autolysate in a 10-ml thin-walled test tube for 2 minutes in a boiling water bath, cooling, centrifuging, and determining the nitrogen content of the clear centrifugate. The total soluble nitrogen content, total N, minus the heat noncoagulable nitrogen, HNC-N, is the heat coagulable nitrogen. Due to evaporation of part of the aliquot during the heating process, the HNC-N was sometimes even higher than the total N, so that trichloracetic insoluble nitrogen content was determined as a rule.

The trichloracetic acid-insoluble nitrogen content was determined by diluting one volume of autolysate with four volumes of 10 per cent trichloracetic acid, and analyzing for nitrogen before and after centrifuging. The former value was used for total N of the autolysate, the latter value was a measure of the T.C.A. soluble nitrogen (that is, organic nitrogenous constituents soluble in 8 per cent trichloracetic acid). The difference between these, the T.C.A. insoluble N, was used as a measure of water-soluble proteins.

The ammonia nitrogen (NH_3-N) was determined by distilling an aliquot of not over 5 ml made alkaline to thymol blue with saturated borax solution into boric acid solution in a Kirk micro-Kjeldahl still.

The phosphate content was determined on an aliquot of the autolysate containing 0.1 to 1.0 mg phosphate by a modification of the method of Sumner (1944). Preliminary experiments with yeast autolysate indicated that a considerable quantity of phosphate was present as a bound compound undetected by this method. The total phosphate liberated was determined by digesting 10 ml or less of the autolysate, containing not over 1 mg of phosphate, with 0.7 ml of 70 per cent perchloric acid until the mixture was completely decolorized. The digest after cooling was treated as in Sumner's procedure. The intensity of the molybdate-blue color produced by reduction with ferrous sulfate was measured in a Klett-Summerson photometer using a red (660) filter.

The nucleic acid released during autolysis was determined by analyzing the cells centrifuged from a 5-ml aliquot of the autolyzed suspension by the method of Di Carlo *et al.* (1949). The optical density of the final hydrolysate at 260 mμ was measured in a Beckman Model DU spectrophotometer using a standard prepared from Eastman Kodak nucleic acid according to Di Carlo and Schultz (1948).

The tyrosine content of the undiluted autolysate was determined by the method of Lugg (1937) as modified by Doty (1941).

Attempts to use the formal titration method of Northrop (1926) for the determination of amino acid nitrogen (NH_2-N) were unsuccessful because of the presence of buffers. The amino acid nitrogen content was determined by a modification of the colorimetric naphthaquinone method of Frame *et al.* (1943). The

color was developed as in the method of Frame *et al.* (1943) but because it was not stable, the per cent transmission at 480 mμ in a Coleman spectrophotometer was measured 45 minutes after the color developed. It was found that transmission increased quite rapidly in the period of 20 to 40 minutes and then only gradually during 40 to 60 minutes, taking the time at addition of acid as zero. On further standing it began to increase again, owing to continued fading, and turbidity developed. A control containing an amount of buffer equivalent to that in the aliquot taken for analysis was used in adjusting the photometer. Glutamic acid was used as the standard for comparison.

Periodically during the incubation period, samples were withdrawn for microscopic examination. The 2-ml aliquots were centrifuged and the sediment examined using the differential staining method of Mills (1941) to determine the percentage of living cells and the method of Shimwell (1938) for the detection of ascospores.

Results

Effect of type of buffer. Previous investigators (Drews, 1936, Haehn and Leopold, 1934, 1937, Schmitt and Gomez Daza, 1944) noted the production of acid and consequent decrease in pH during autolysis in some cases, and increase in pH value in others. The acid shift in pH occurred at higher initial pH's, and the alkaline shift occurred in more acid regions. The acid shift was more pronounced in glycogen-rich than in glycogen-poor cells and the alkaline shift was more pronounced in old or protein-rich cells. To reduce this effect, autolysis of fresh baker's yeast was carried out in sterile, non-

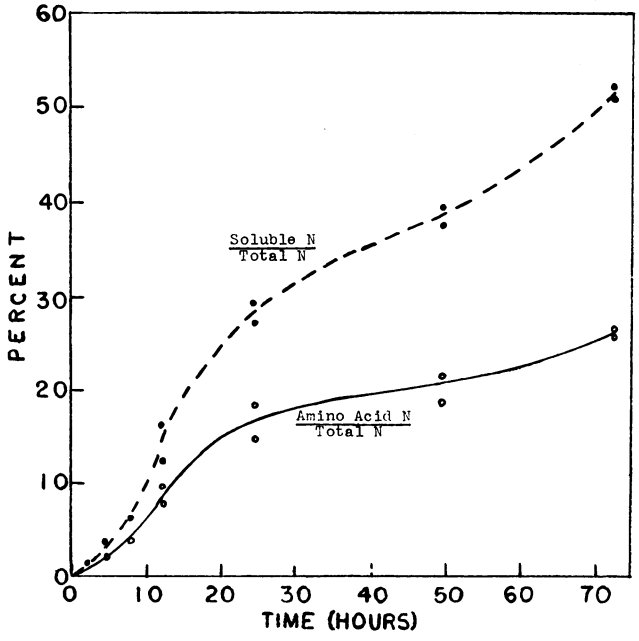

FIG. 1. Autolysis of baker's yeast in 0.5 M citrate at pH 5 and 53 ± 1 C.

nutrient 0.5 M phosphate, citrate and arsenate buffers at pH 5.0 and 53 C. Duplicate lots were removed at intervals of 0, 2, 4, 8, 12, 48 and 72 hours and analyzed for total organic nitrogen content before centrifuging and for pH, amino nitrogen, soluble nitrogen, and heat noncoagulable nitrogen after separation of the cells. The total nitrogen content varied from 2.97 to 3.18 mg per ml in the phosphate buffers, 3.28 to 3.45 in the citrate buffers, and 2.67 to 3.15 in the arsenate buffers. The pH varied from 4.18 to 5.78 in the phosphate buffers, 4.97 to 5.18 in the citrate buffers, and 4.68 to 5.58 in the arsenate buffers.

In all three buffers, there was a sharp initial drop in pH up to 4 hours. The pH then sharply increased up to 12 hours, and then very slowly decreased from 12 to 72 hours. In all cases, the final pH after 72 hours was greater (more alkaline) than when the autolysis began.

It was quite striking (especially in phosphate) that relatively large changes in pH occurred during the first 12 hours of autolysis, for during this time the autolysis was just beginning. From 12 to 72 hours, when the bulk of the products of autolysis was formed, there was a comparatively small change in pH. During the first 12 hours of autolysis, changes took place in the cell wall or membrane to allow the products of autolysis to diffuse into the surrounding liquid, and this, rather than the products themselves, may be the cause of such large fluctuations in pH.

The rate of change in soluble nitrogen and in amino acid nitrogen content, expressed as per cent of the total nitrogen present in the suspension, is shown for the citrate buffer in figure 1. The soluble and the amino nitrogen content increased slowly during the first initial period of about 4 hours, then quite rapidly during the next 20 hours and more slowly thereafter. The soluble nitrogen content, however, increased more rapidly than did the amino acid nitrogen content. Autolysis in the phosphate buffer (figure 2) was similar to that in the citrate buffer but in the arsenate buffer (figure 3) the increases in both the amino N and the soluble N were more rapid after an initial lag period. The per cent of total nitrogen converted to soluble nitrogen in the citrate, phosphate, and arsenate buffers, respectively, after 12 hours in duplicate samples were: 12.4, 16.2; 15.3, 15.6; 9.4, 14.7. For amino N after 12 hours the conversion percentages were: 7.46, 9.08; 10.3, 10.9; 7.0, 10.3. After 72 hours the conversion percentages for amino N in the three buffers were, respectively, 26.2, 26.8; 27.3, 34.3; 45.7 and 52.7. After 72 hours the conversion percentages for soluble N were: 50.7, 51.8; 44.4, 50.8; 65.8 and 70.4. Autolysis in the arsenate buffer was about 50 per cent greater than in the phosphate or citrate buffer after 72 hours. In the arsenate buffer, about 50 per cent of the cell nitrogen was converted into amino acids, whereas, in the phosphate and citrate buffers, the conversion was less than 30 per cent.

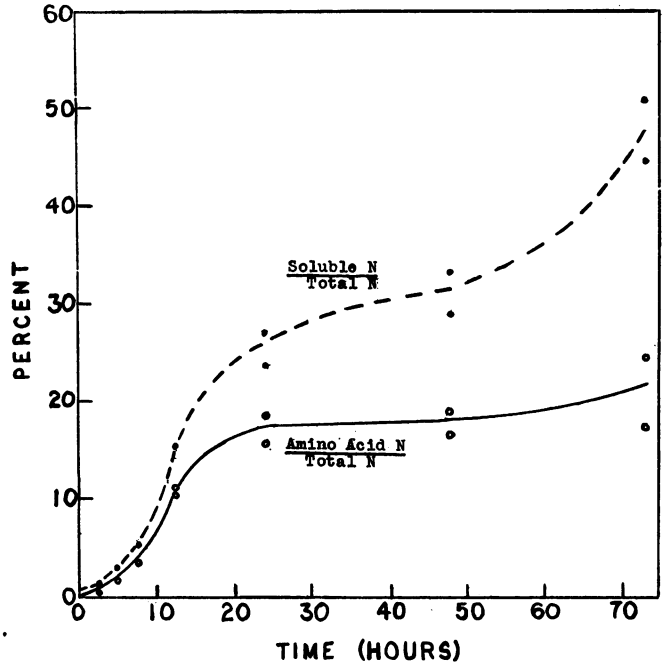

FIG. 2. Autolysis of baker's yeast in 0.5 M phosphate at pH 5 and 53 ± 1 C.

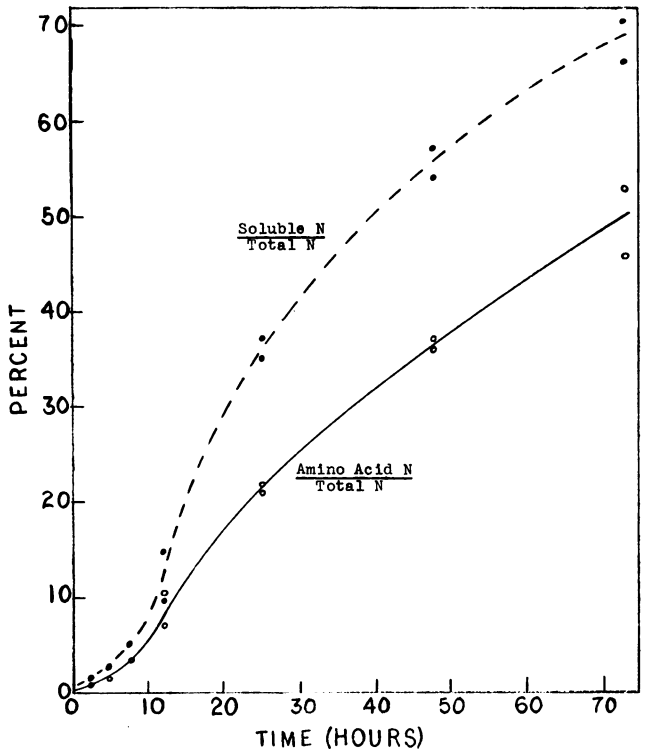

FIG. 3. Autolysis of baker's yeast in 0.5 M arsenate at pH 5 and 53 ± 1 C.

Effect of buffer concentration. Baker's yeast was autolyzed for 3 days at 55 to 56 C in 0.1 and 0.5 M citrate adjusted to different pH's within the pH range of 3.1 to 4.2 (figure 4). At the lowest pH investigated (3.13 to 3.17), the amounts of amino acid nitrogen and total soluble nitrogen found in the autolysate were about 100

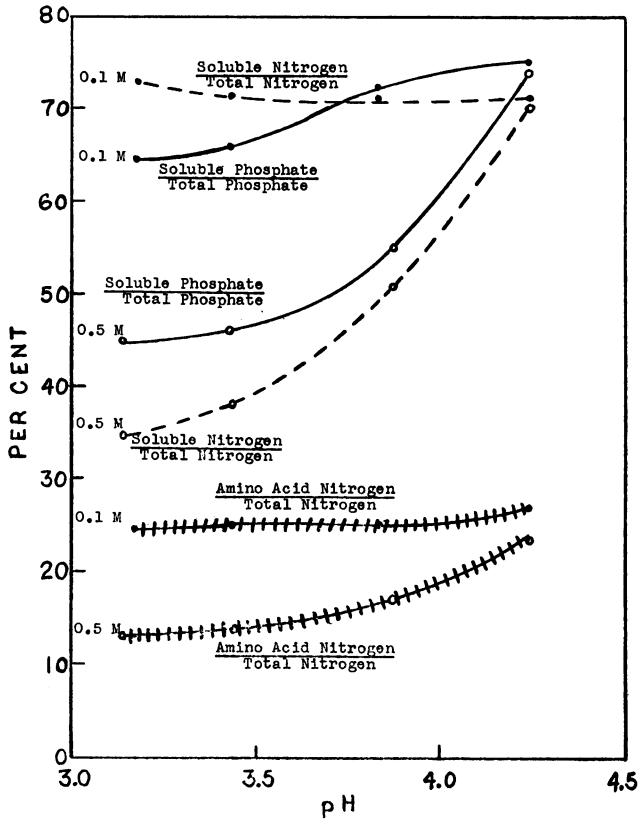

FIG. 4. Autolysis of baker's yeast at 55–56 C in 0.1 M and 0.5 M citrate and different pH's.

per cent greater in 0.1 M citrate than in 0.5 M citrate, while phosphate was about 50 per cent greater. At the highest pH (4.22 to 4.23) investigated, the above substances were not affected by the citrate concentration.

The ratio of amino acid nitrogen to total soluble nitrogen was found not to depend on buffer concentration at each pH, indicating that the action of peptidases was not affected by citrate concentration. In other words, no matter how little or how much nitrogen was elaborated into the liquid surrounding the cells, the relative amount of amino acids found did not vary. This suggested that the depressing effect of 0.5 M citrate at lower pH's may be due to suppression of diffusion of products from within the cells to the surrounding media by the higher citrate concentrations, or to the inability of 0.1 M citrate to hold the pH down to the same level which 0.5 M citrate maintained.

Effect of cell density. The possibility that there may be a relation between the density of cell suspensions and degree of autolysis was investigated. Five different suspensions of various cell densities (0.82 to 7.5 mg total nitrogen per cc) in citrate buffer (0.1 M at pH 4.92) were stored for 72 hours at 53 to 54 C.

Analysis of the autolysate showed an alkaline shift in pH which increased with cell concentration as much as 0.40 units. Cell density appeared to have no effect on the degree of autolysis. The products of autolysis

TABLE 1. *Effect of cell density on autolysis*

TOTAL N	pH	PER CENT CONVERSION OF TOTAL N			PER CENT CONVERSION OF TOTAL PO_4 INTO SOLUBLE PO_4
		Into soluble N	Amino N	NH_3 N	
mg/ml					
0.828	5.02	38.4	17.6	1.1	26.7
2.11	5.08	36.9	17.9	0.9	29.2
4.27	5.15	37.0	17.9	0.95	26.9
6.37	5.24	37.8	18.3	0.95	26.8
7.64	5.31	37.6	19.5	0.95	26.6

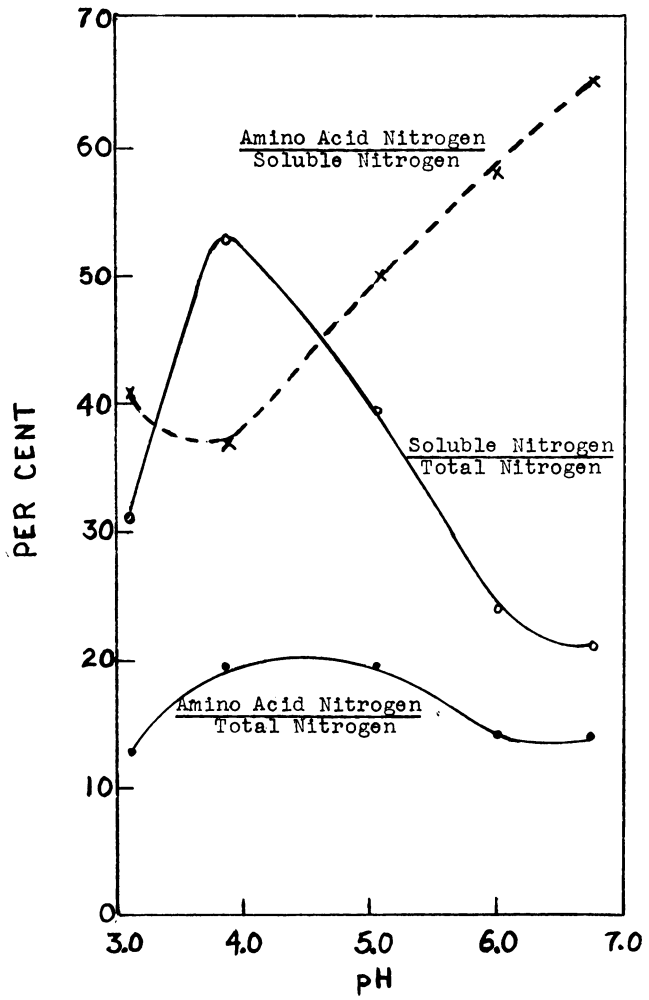

FIG. 5. Autolysis of baker's yeast in 0.5 M citrate at different pH's after 24 hours at 53 C.

were liberated in quantities proportional to the initial cell concentration (table 1). By diluting portions of the total suspensions after autolysis to a common cell density (0.820 mg total nitrogen per ml), analyzing the autolysate, and then correcting for dilution, it was possible to show that cell concentration had no effect on the degree of autolysis. This dilution to a common cell density prior to analysis eliminated the possible interference of cell concentration in expressing the results.

The amount of ammonia nitrogen that was formed during autolysis under these conditions was quite low (only about 1 per cent of the total cell nitrogen). Amino acids accounted for about 50 per cent of the nitrogen in the autolysate. The amount of protein nitrogen present was even less than the amount of ammonia nitrogen.

Effect of pH. Suspensions of yeast in sterile 0.5 M citrate buffered at pH's 3.05, 4.03, 5.01, 6.02, and 6.95, respectively, were allowed to autolyze for 24 hours at 53 C. The observed effect of pH on the nitrogenous products of autolysis is shown in figure 5. The data indicate that pH had a marked influence not only on the degree of autolysis but also on the nature of the products formed. The optimum pH for autolysis under these conditions, with respect to the amount of total nitrogen and amino acid nitrogen released from cells, was about pH 4.0. There was an alkaline shift in pH (after, as compared with before, autolysis) from pH 3 to 6; however, the samples initially at pH 6.74 shifted slightly towards the acid side after autolysis. This was previously observed by Drews (1936). It was also noted that the pH's of the suspensions were higher than those of the supernatant liquids obtained by centrifuging the suspensions; thus it is important to be consistent in the method of determining pH. Jenny *et al.* (1950) have pointed out the difficulties involved in measuring pH of colloidal solutions.

The proportion of the total soluble nitrogen in autolysate as amino acid nitrogen increased from about 40 per cent at pH 3 to 65 per cent at pH 7. Although the optimum pH for the formation of amino acid nitrogen was near 4.0, it is apparent that the enzymes (peptidases) producing these acids acted on the degradation products of the yeast protein, rather than the protein itself. In other words, the previous action of other enzymes (proteinases) on the yeast protein allowed for subsequent peptidase activity on the products of protein degradation.

Effect of toluene. Since earlier work regarding the effect of antiseptics on yeast autolysis had been carried out in unbuffered solutions, it was of interest to determine the effect that toluene has on the autolysis of baker's yeast in solutions buffered near the optimum pH for autolysis. Haehn and Leopold (1935) previously observed that 5 per cent toluene had only a very slight effect on autolysis, as compared with the normal antiseptic-free autolysis.

Parallel experiments (with and without 1 per cent toluene) were conducted in 0.1 M citrate at pH values of 3.46, 4.04, 4.50 and 4.98. Analyses were made after 3.5, 4.0, 4.5 and 5.0 hours of autolysis. It was found that toluene had no effect on the final pH after autolysis or on the degree of autolysis as measured by release of total nitrogen or phosphate from the cells. The portion of the total nitrogen in the autolysate that was amino acid nitrogen was about 10 per cent less in the samples autolyzed in the presence of toluene, indicating a slightly inhibitory effect of toluene on peptidase activity.

Effect of temperature. To obtain data on the effect of temperature on the rate of autolysis at several pH values, samples of yeast were autolyzed for 72 hours at temperatures of 34 to 36 C, 45 to 46 C and 54 to 55 C in 0.5 M citrate at pH's of 4.12, 4.40 and 4.64. The final pH, total soluble nitrogen, amino acid nitrogen, and total phosphate content of the cell-free centrifugate were determined. Figure 6 shows the per cent of soluble nitrogen found at various pH values and incubation temperatures. Autolysis was almost completely absent at 35 C. The percentage of conversion of total N at this temperature at all pH values into soluble N was about 4 per cent, into amino N about 2.5 per cent and the conversion of total phosphate into soluble phosphate was 4 per cent at pH 4.1, 2.5 per cent at pH 4.4, and 1.3 per cent at pH 4.64. The optimum temperature for autolysis was 45 C as measured by the amount of nitrogen, phosphate, and amino N found in the autolysate. The optimum pH for autolysis both at 45 and 55 C was 4.4 with respect to nitrogenous materials liberated. A pH higher than 4.64 appeared to be optimum for the release of phosphates. At the higher temperatures more of the original phosphate was liberated than of the original nitrogen. At 45 C and pH 4.4, over 75 per cent of the total nitrogen was present in the autolysate as soluble nitrogen, and about 30 per cent of the total N was present as amino N. Under the same conditions about 85 per cent of the initial phosphate content was present as soluble phosphates.

Effect of viability. To determine the relation of cell viability to autolysis, yeast suspended in sterile 0.5 M citrate at pH 4.38 was incubated for 24 hours at 34 C and 45 C and for 48 hours at 34 and 45 C. At the conclusion of the specified period of storage, the number of viable cells was determined by methylene blue staining and by the pour plate method. The autolysate was analyzed for soluble nitrogen and phosphate. The effect of temperature on autolysis was found to be more than merely killing the cells. The samples stored at 34 C contained only 8 per cent viable cells after 24 hours; however, only 3.2 per cent of the total cell nitrogen and 1.3 per cent of the total cell phosphate appeared in the autolysate. Those stored at 45 C contained no viable cells after 24 hours, while 41 per cent of the total cell nitrogen and 76 per cent of the total cell phosphate appeared in the autolysate. The same general relation held for the samples incubated for 48 hours. In other words, storage conditions that killed yeast cells did not necessarily produce autolysis, while conditions necessary to cause extensive autolysis were so severe that none of the cells were viable after 24 hours.

Stimulation of autolysis. Since it was shown that a 24-hour storage at 34 C killed over 90 per cent of the cells without producing any appreciable amount of autolysis, it was thought possible to stimulate these dead cells into autolyzing at the lower temperature.

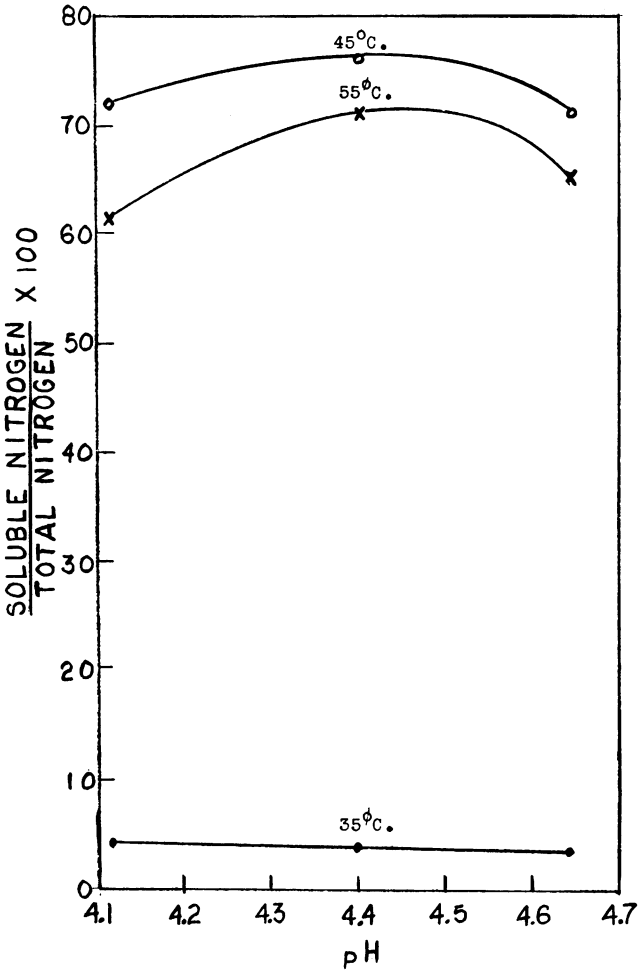

FIG. 6. Autolysis of baker's yeast in 0.5 M citrate at different pH's and temperatures.

Attempts to stimulate autolysis at 34 C by means of added chemicals or pretreatment of the cells met with some success. A suspension incubated in 0.01 M dinitrophenol at 34 C exhibited nine times greater autolysis than the control (34 C and no dinitrophenol) and over half that of a sample autolyzed at 45 C. Since it had been previously established that these conditions of temperature (34 C), time (24 hours), and pH (about 4.4), killed the cells but did not activate the autolytic enzymes, it follows that the effect of DNP was to stimulate these enzymes and thus increase the degree of autolysis. Sodium fluoride and potassium cyanide exhibited only a slight activating influence at the lower temperature.

Of the other methods used, pretreatment with a 2 per cent Tide (a Proctor and Gamble detergent) solution was more effective at 60 C than when used at room temperature and also more effective than an initial washing in water at 60 C. The locus of action for Tide was probably the cell wall. Sodium cholate (a detergent) has been shown to remove the gram complex from cells (Stacey and Webb, 1948). Tide probably acts by

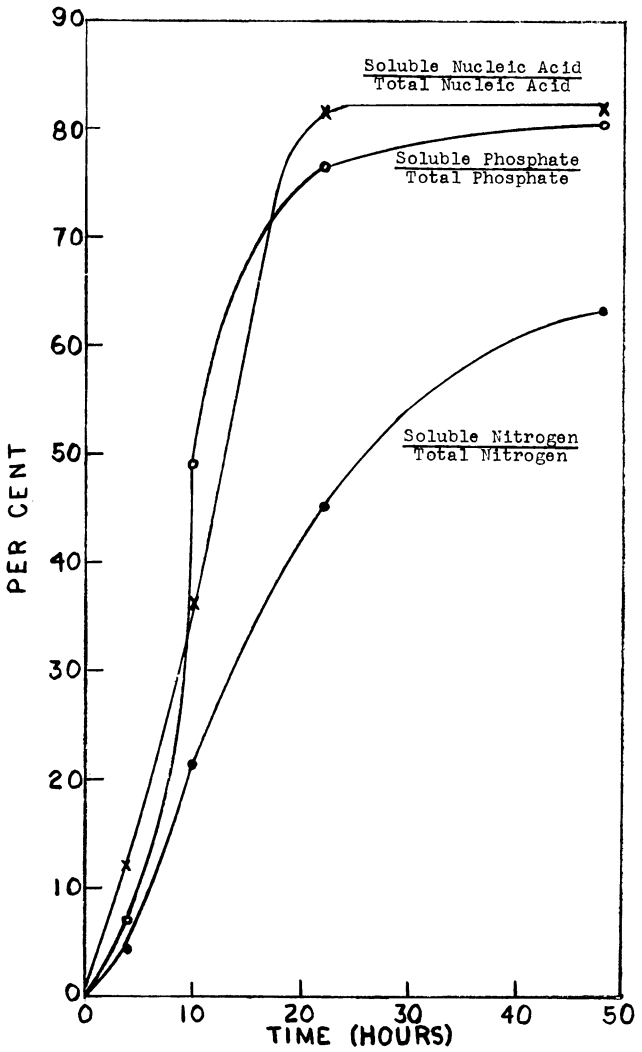

FIG. 7. Release of nucleic acid, phosphate, and nitrogen during the autolysis of baker's yeast.

removing ribonucleic acid and better preparing the yeast cells for autolysis.

At 45 C it was found that 10^{-2} M sodium azide produced a 41 per cent increase in the degree of autolysis, as compared with the control. Dinitrophenol increased autolysis about 17.5 per cent, while sodium arsenate showed only a 1.7 per cent increase over the control. At 33 C DNP had the greatest influence, followed by azide and then arsenate.

Release of nucleic acid during autolysis. Nucleic acids are closely associated with the living processes of yeast and their content in yeast roughly parallels the cell's synthetic activity. Nucleic acid release during autolysis was investigated and found to parallel roughly the release of phosphate (figure 7). The nitrogenous materials in the cell were released at a much slower rate than both the phosphate and nucleic acid.

The yeasts in the different samples were tested for their gram-staining ability. One hundred per cent of the cells in samples stored for 0, 4 and 10 hours were gram positive. With the latter two samples, it was quite difficult to get reproducible results since the cells were not uniformly gram positive or gram negative. The lack of uniformity was especially noted in the sample autolyzed for 48 hours; some gram-negative cells were observed to have gram-positive areas within them. The staining results indicated that changes in the nucleic acid content were occurring, but not to the extent borne out by chemical analysis (82 per cent of the nucleic acid lost from the cell after 22 hours).

DISCUSSION

Yeast cells suspended in a nutrient-deficient buffer and incubated at temperatures of about 50 C die and undergo autolysis. During autolysis a yellowish hue appears in the solution surrounding the cells. As autolysis proceeds, the intensity of the color increases and after 72 hours the solution becomes golden yellow in color. Microscopic examination shows granulation and occasionally globule formation in the cytoplasm. Occasionally budding and spore formation is observed. Under no conditions is lysis of the cell wall observed. No cell wall fragments were observed even when baker's yeast was ground with calcium carbonate in the presence of chloroform (180 grams yeast, 11 grams $CaCO_3$, 11 ml chloroform) and stored until the mass was completely liquefied. Shihata and Mrak (1951) also failed to observe lysis of cell wall of baker's yeast during digestion by *Drosophila*. During autolysis, partial hydrolysis of the cell proteins occurred in the cytoplasm but both heat-coagulable or trichloracetic acid-precipitable proteins as well as soluble proteins were released into the medium. Since the proteins accumulated at concentrations higher than amino acids, it appears that hydrolysis into amino acids occurred subsequently. The separation of the two processes by variation in type of buffer, pH, temperature, and antiseptic clearly indicates the essential difference between them. Whether amino acid formation is exocellular or endocellular is still not clear but there is some evidence that proteolytic enzymes as well as polypeptides are released during autolysis.

Nitrogenous compounds are known to be excreted by the yeast cell in the growing phase as well as during autolysis, and this excretion is greater in young actively growing cells and in nitrogen-rich cells. Nitrogen excreted during autolysis is strongly dependent on temperature, but not that excreted during growth. It was established early that autolysis of yeast is never complete and that the products of autolysis inhibit the process. This fact led Iwanoff (1914, 1921) to postulate that synthesis of proteins could occur in the later stages of autolysis. Protein synthesis may occur but this has not been established. It is likely that in the initial stages the yeast proteins are hydrolyzed to low molecular weight polypeptides by the action of yeast pepsin

and trypsin having pH optimum at 1.8 and 7.8, respectively, according to Hecht and Civin (1936). The yeast erepsin (a mixture of exopeptidases) then removes the terminal amino acids from these polypeptides. Phosphate liberation occurs more rapidly and more extensively during autolysis than does protein hydrolysis. Nucleic acids accumulate almost as rapidly as phosphates and there is some evidence that change in gram stain also is involved. The actual mechanism of proteolytic changes occurring during autolysis, however, is still unknown.

Summary

The autolysis of fresh compressed baker's yeast was investigated under starvation conditions in buffered solutions which were found to minimize but not completely eliminate changes in pH during autolysis. The nature, as well as the concentration, of buffer was found to influence autolysis, especially regarding shifts in pH during autolysis. Extensive fluctuations in pH could not be attributed to the formation of autolysis products. Since the bulk of autolysis occurred after the first 12 hours, when the pH changes were most pronounced, it appeared that changes which took place in the cell wall or membrane caused these large pH shifts. Microscopic examination of baker's yeast suspended in 0.1 M citrate and phosphate failed to reveal any lysis of the cell wall during autolysis. The lack of cytase activity was also noted in baker's yeast which was completely liquefied by the action of chloroform. The course of autolysis in different buffers at 52 to 54 C appeared to follow a sigmoid curve. A latent period was noted during the first 8 hours; followed by an abrupt release of autolysis products during the next 16 hours; little change during the following 24 hours and then a slight increase after an additional 12 hours. Phosphate and nucleic acids appeared to be released more rapidly than the cell's nitrogenous constituents. Although the nucleic acids were lost quite readily and extensively during autolysis, a corresponding loss in gram staining ability was not observed. The proportion of the total nitrogen in the autolysate that was amino acid nitrogen was affected by the pH of autolysis, being optimal near neutrality due to enhanced peptidase activity. The substrates determining the amount of amino acids to be found were the degradation products of the yeast protein, rather than the protein itself. Cell density had no effect on the degree of autolysis. Autolysis in the presence of 1 per cent toluene was almost the same as in an antiseptic-free media, although toluene did inhibit peptidase activity about 10 per cent. The autolysis of baker's yeast was greatly influenced by temperature and the results indicated that death must precede autolysis, although it does not necessarily follow that dead cells will autolyze. Autolysis was stimulated at low temperatures (about 33 C) in the presence of 0.01 M DNP and by pretreating the cells at 60 C with a 2 per cent solution of Tide; however, more dilute solutions of DNP as well as 0.01 M solutions of sodium azide, sodium arsenate, sodium fluoride, or potassium cyanide failed to activate autolysis at this temperature.

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